

REVIEW

Wheat gluten protein and its impacts on wheat processing quality

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Abstract Before the advent of the wheat genomic era, a wide range of studies were conducted to understand the chemistry and functions of the wheat storage proteins, which are the major determinants of wheat flour the suitability of wheat flour for various end products, such as bread, noodles and cakes. Wheat grain protein is divided into gluten and non-gluten fractions and the wheat processing quality mainly depends on the gluten fractions. Gluten provides the unique extensibility and elasticity of dough that are essential for various wheat end products. Disulfide bonds are formed between cysteine residues, which is the chemical bases for the physical properties of dough. Based on the SDS-extractability, grain protein is divided into SDS-unextractable polymeric protein (UPP) and SDS-extractable polymeric protein. The percentage of UPP is positively related to the formation of disulfide bonds in the dough matrix. In the wheat genomic era, new glutenins with long repetitive central domains that contain a high number of consensus hexapeptide and nonapeptide motifs as well as high content of cysteine and glutamine residues should be targeted.

Keywords wheat gluten, consensus motifs, disulfide bonds, SDS-unextractable polymeric protein, glutenins, gliadins, processing quality, storage protein

1 Wheat grain protein classification

Bread wheat grain proteins are generally divided into gluten and non-gluten proteins (Fig. 1). Both gluten and non-gluten proteins are located mainly in the embryo, aleurone layer and endosperm of the grain, with the endosperm containing most of the gluten. The gluten protein fraction accounts for about 85% of total grain protein. Based on the solubility in aqueous alcohols and acid solution, gluteins are divided into polymeric glutenins

and monomeric gliadins in a proportion of 40% and 60%, respectively^[1]. According to the molecular weight distribution, glutenins are classified into high molecular weight (70000–90000 Da) glutenin subunits and low molecular weight (20000–45000 Da) glutenin subunits (HMW-GS and LMW-GS), which account for 40% and 60% of glutenin composition, respectively. Based on the order of mobility on electrophoresis at low pH, monomeric gliadins are classified into α/β -, γ - and ω -gliadins, which represent approximately 55%, 30% and 15% in gliadin fractions, respectively^[2,3]. The non-gluten protein includes albumins (water-soluble protein) and globulins (salt-soluble protein). These are mainly biochemical functional proteins such as chaperones and enzymes, which regulate the accumulation and synthesis of storage proteins, and grain growth^[4,5].

2 Wheat gluten protein variations

Glutens are the major constituents of bread wheat grain storage proteins and constitute 85% of the total. They are mainly responsible for the processing quality of wheat dough and were among the first proteins isolated and studied by human beings. Their biological function is to provide carbon, nitrogen and energy sources for seed germination and seedling growth. Mutations or silencing of such genes are not lethal for the plant, so the evolutionary selection pressure on these genes is much lower than for functional genes^[6]. As a result, these genes can accumulate more mutations, making them ideal model molecules for studying a range of biological fundamental processes^[7–10]. It is worth noting that past work has been primarily focused on the applied aspects of using these proteins to increase wheat end product quality. Their value in theoretical research has been largely ignored. So far, only Zhang et al.^[8] has reported a biological function other than the storage function for gluten, an antifungal function of a newly identified special gluten protein, namely avenin-like protein, which is quite similar to the gliadins in

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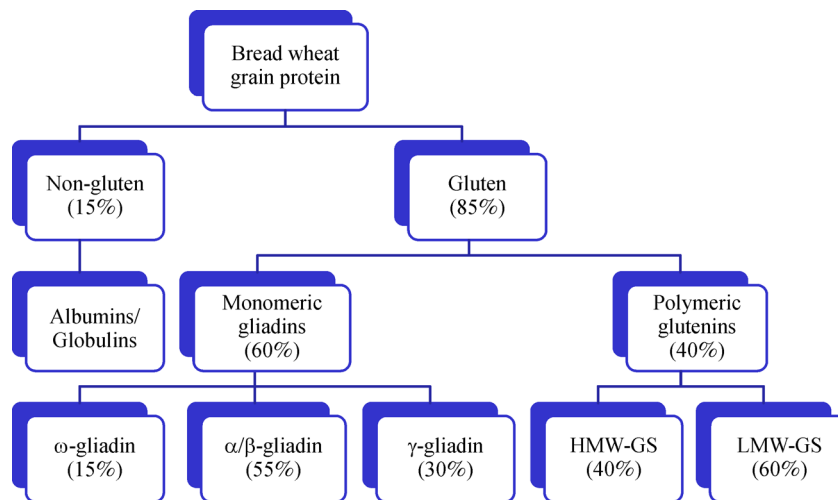


Fig. 1 Classification of wheat grain proteins

structure. Until now, extensive studies have provided substantial information about the relationship between gluten structures and properties in relation to end product quality. The first systematic study was conducted by Osborne^[2], who developed a classification for cereal-seed proteins based on their sequential extraction and differential solubility. Four different groups were classified, including (1) albumins, soluble in water and dilute buffers, (2) globulins, not soluble in water but soluble in saline solutions, (3) gliadins, soluble in 70% to 90% ethanol, and (4) glutenins, soluble in dilute acid or alkali. The two distinct groups of the gluten polymer that were classified reflected their solubility in 70% ethanol, namely glutenins and gliadins^[11,12]. The gliadins are single polypeptide chains and the glutenins are multichained structures of polypeptides that are held together by disulfide bonds. The high molecular weight of these polymeric structures is responsible for their partial insolubility and for their contribution to food-product quality. Therefore, the classification of these proteins into monomeric and polymeric forms is a good indicator of their functional properties^[13].

In most dicotyledonous, and some monocotyledon seeds, the globulin types predominate in the seed. However, in the Triticeae (wheat, barley and rye) the major portion of seed proteins are not globulins, but classes of protein characterized by regular repetitive domains with unique and fundamental functional features^[14,15]. These proteins are glutenins in wheat and variation in them either quantitatively or qualitatively has major effects on end product quality^[16–18]. It is worth noting that although some water-soluble proteins are also found present in the dough gluten matrix and have some impacts on wheat processing quality^[19], the glutenins and gliadins are still the dominant proteins in defining wheat processing quality. The predictive power of glutenin subunit proteins for flour processing properties has been demonstrated for dough

rheological properties, such as dough extensibility and elasticity^[18,20,21]. Strong dough will form a cohesive mass that has resistance to extension and can retain stability during mixing. Such dough is able to hold the gas produced during fermentation within evenly distributed discrete cells in the dough structure. This results in a loaf crumb in which the gas cells are of regular size and even distribution. Such a crumb structure appears light in color, fine and silky in texture, both highly desirable quality attributes. Soft gluten will allow the gas cells to expand excessively during fermentation, causing their walls to collapse and the cells to coalesce together, resulting in the bread having an open texture with a coarse wall structure^[22].

The investigation of glutenin proteins in relation to dough properties have indicated two key variables: (1) the nature of the protein allele^[16,23] and (2) the level at which the respective allele is expressed^[24,25]. The control of the level of expression of seed storage protein has been well studied^[26–28]. Studies of the regulatory mechanism of gene expression performed by Wanous et al.^[29] revealed that 15 chromosome arms had significant effects on Glu-B1-1, 19 on Glu-B1-2, 20 on Glu-D1-1 and 25 on Glu-D1-2. In addition, the orthologous loci (both x-type and y-type HMW-GS) are influenced by the same regulatory systems and there is less correlation between paralogous genes, although considerable conservation was observed at this level. However, there are chromosome sequences not coding for any structural genes that affect the expression of seed storage protein encoding genes in wheat. Chromosome 1D and 2A sequences can affect the expression of gliadin genes located on chromosome 6A and 6D, respectively^[30,31]. The DNA structures of most of the glutenin genes have been determined^[24,32–35]. The prolamins forming the polymer are mainly HMW-GS and LMW-GS, while the monomeric (polymer non-participating) prolamins are called α/β -, γ -, and ω -gliadins. It is

worth noting that the α - and ω -gliadins have been confirmed to be the cause of human celiac disease and wheat intolerance, while the glutenins are classified as nontoxic, weakly toxic or not as toxic as gliadins^[36]. The variation in celiac disease epitopes in the α -gliadin gene family of hexaploid wheat has been extensively studied^[37–39].

HMW-GS proteins are a major determinant of gluten elasticity through promoting the formation of larger glutenin polymers and thus are key factors for breadmaking^[40]. They are encoded by the *Glu-1* loci *Glu-A1*, *Glu-B1* and *Glu-D1* that are located on the long arms of chromosome 1A, 1B and 1D, respectively. Each locus includes two genes linked together encoding two different types of HMW glutenin subunits, x-type and y-type subunits^[41–43]. The x-type subunits generally have a higher molecular weight than that of y-type subunits^[44]. Payne and Lawrence^[13] summarized the number of alleles at *Glu-1* loci, three allelic forms for *Glu-1A*, 11 alleles for *Glu-1B*, and six alleles for *Glu-1D*. More alleles have been identified since as reported by researchers^[45–47]. Most alleles in modern hexaploid wheat cultivars emerged before hexaploid wheat was formed, only By18 subunit has been confirmed to have emerged after hexaploid wheat formation^[48]. Many gluten alleles did not enter into the hexaploid wheat during evolution or have been discarded through modern breeding^[7,35]. Although six genes exist for HMW glutenin subunits, due to gene silencing, most hexaploid wheat cultivars possess three to five HMW glutenin subunits^[33]. All hexaploid wheat genotypes contain at least Bx, Dx and Dy subunit in their endosperm, while most cultivars also contain a By subunit and an Ax subunit. The gene encoding Ay subunit is usually silent^[33,49]. By introducing an active Ay gene into Australian common wheat cultivars, Roy et al.^[50] found significant positive effects of the Ay subunit on processing quality. Other allelic effects associated with HMW glutenins have been well documented. The one most reported and used is Dx5 + 10^[16,51,52]. Increased dough strength can also be achieved through an increase in expression of the Bx7 subunit, which results in approximately 130 BU (Brabender units) over the average of the other alleles at that locus^[20,24]. It has been observed that the overexpression of *Glu-1Bx7* improves dough strength by stabilizing the gluten network, whereas *Glu-1Bx17* and *Glu-1Dx2* do not^[53]. Compared to the normal Bx7 gene, the gene conferring Bx7 overexpression has an 18 bp insertion in the central repetitive domain, a 43 bp indel of the 5'-region and the left and right junctions of the LTR retrotransposon borders and the duplicated segment, and has been used to develop PCR markers as well as high throughput KASP markers for differentiating these two genotypes^[54,55]. Other allelic variants also have differential effects on dough quality, e.g., *Glu-B1* subunits 17 + 18 are associated with strong dough while subunits 20x + 20y are associated with weak dough^[56]. Statistical analysis of large numbers of durum wheat genotypes confirmed the

strong correlation of γ -gliadin 45 with good processing quality and γ -gliadin 42 with poor processing quality. Moreover, loaf volume during the baking process has a negative relationship with acetic acid-soluble glutenin and a positive relationship with acetic acid-insoluble glutenin^[11]. For extensibility, Cornish et al.^[56] suggested this was a more complex trait involving other parameters such as LMW-GS and gliadin compositions. However, gliadins appear to be less important in determining bread quality, and the addition of gliadins or the overexpression of certain gliadins can reduce dough strength^[57]. Wieser^[58] found that hydrated gliadins have little elasticity but contribute to the viscosity and extensibility of the dough system, whereas, hydrated glutenins are responsible for both cohesive and elastic properties.

3 Gluten protein structure and component variations and their impacts on dough quality

Typically, the structure of gluten protein is determined by three general domains, one central domain rich in a repetitive structure constituting a β -reverse turn and two terminal α -helix domains^[59]. A long repetitive domain of the glutenin is considered to have a positive influence on wheat flour quality due to the formation of more β -reverse turn structures^[40,60]. The proportion of the consensus hexapeptides and nonapeptides in the repetitive domain also affect dough quality. Masci et al.^[61] reported that a large and regular repeated sequence domain increases the viscosity and elasticity of doughs through intermolecular interactions. Wang et al.^[25] reported that the hexapeptide motif is more important than the nonapeptide motif. In terms of secondary and high order structure, gluten protein can aggregate to form a complex protein network through disulfide bonds during the dough mixing process. In general, most y-type subunits contain seven cysteines (five in the N-terminal domain and one in each of the repetitive and C-terminal domains), the x-type subunits possess four cysteines (three in the N-terminal domain and one in the C-terminal domain)^[62,63]. However, different subunits possess different numbers of cysteines. For example, the subunit 1Dx5 has five cysteines (three in the N-terminal domain and one in each of the repetitive and C-terminal domains) and 1Dx2 only has four cysteines, meaning that 1Dx5 possesses positive allelic effects over 1Dx2 on dough strength^[64,65]. Also, Ax2*B is a novel variant of the Ax2* subunit, which contains an extra cysteine residue located in the middle of its repetitive domain, exerts a positive effect on the gluten properties^[66]. In contrast, Bx14 and Bx20 subunits have reduced numbers of cysteine residues in their N-terminal domains and usually have negative effects on dough strength^[64]. Apart from the disulfide bonds, Gilbert et al.^[67] found that hydrogen bonds formed through glutamine can stabilize the polymeric structure of glutenin. Studies have revealed that a

high content of glutamine of both HMW and LMW glutenins have positive effects on dough quality^[68]. Extra covalent bonds and intermolecular crosslinks of tyrosines in the form of isodityrosine or dityrosine were found to form during the dough mixing and baking process among gluten proteins of wheat at the site of repeats of pairs of tyrosine residues throughout the central repetitive domains^[59]. Oxidizing agents, such as ascorbic acid, azodicarbonamide, and potassium bromate, can facilitate the formation of dityrosine or isodityrosine during the baking processing. Therefore, tyrosine is also important for the maintenance of secondary, tertiary or quaternary structure of gluten despite being present at a relative low proportion (3%–5%). Cysteine, glutathione, butylated hydroxytoluene and other reductive agents on the other hand are capable of not only cleaving disulfide bonds but also inhibiting tyrosine bond formation via their free radical scavenging activities^[62,69]. However, Peña et al.^[70] indicated that crosslinks between tyrosine residues appear to be few and of little importance in the structure of the gluten network compared to the disulfide bonds formed between cysteine residues. Less than 0.1% of the tyrosyl residues participate in the crosslinks by forming dityrosine, isodityrosine, trityrosine and pulcherosine, and is determined by the number of tyrosyl residues in the central repetitive domain of glutenins. Of those, dityrosine residues are only of minor importance in the structure of wheat gluten^[71]. The large gluten polymers consisting of HMW-GSs, LMW-GSs and other relevant proteins determine the end-use value of wheat flour by affecting dough mixing and gluten formation. However, there are no definitive structural differences among different glutenin subunits which show a close relationship with their ability to form gluten.

Gluten component variations also have a significant impact on dough physical and physicochemical properties. The classic interaction network of gluten involves the backbone formed by HMW-GSs joined by LMW-GSs and gliadins through disulfide bonds and noncovalent bonds, respectively^[72]. Among them, the x-type HMW-GSs confer wheat gluten strength and the ratio of tenacity/extensibility (P/L), and LMW-GSs, together with gliadins, function as solvent which modifies the rheological properties of dough either by interfering with the polymerization of HMW-GS, or by altering relative amounts of different glutenin subunit types^[70]. In detail, W was strongly relevant to the quantity of x-type HMW-GS. This has been confirmed by Halford et al.^[73] who showed that wheat carrying the active alleles of *Glu-A1* (containing Ax1 or Ax2* subunit) can produce higher strength gluten and this was further supported by the positive effects associated with an active y-type HMW glutenin alleles encoded by *Glu-A1* (containing Ay subunit). Besides, a positive correlation existed between rheological parameters W and P/L and the total quantity of x-type HMW-GSs, while a negative correlation was found between P/L

and gliadins/HMW ratio and LMW/HMW ratio^[53]. As Ahmad et al.^[3] proposed, correlations exist between gliadins and certain rheological variables. Therefore, there must be a balance between different types of proteins for the formation of the gluten network and the HMW-GSs contributing to the formation of gluten network, and especially x-type glutenin subunits have an important role in the polymerization, whereas gliadins interfere with the polymerization. Furthermore, Uthayakumaran et al.^[74] found a relationship between extensibility and gliadin quantity and a negative correlation between LMW-GSs quantity and P/L results in an extensible property. These results indicate that the quantities of different gluten protein fractions are more important than the types of alleles available^[53]. A newly characterized storage protein class, avenin-like proteins, that is similar to gliadin in structure with high number of cysteine residues was found to have positive effect on dough strength^[75].

Different compositions of wheat storage proteins confer different dough physical properties that are required by different end products^[76,77]. For example, pasta making requires dough with high gluten strength, but dough for biscuit making needs low gluten strength with high extensibility. Breadmaking needs moderate gluten strength and high extensibility dough, while noodle making needs dough with a balance of gluten strength and extensibility in order to protect dough from tearing during the manufacturing process. In addition, confectionery products such as cake and cookies need flour with weak gluten^[78]. Therefore, it is the balance between elasticity and extensibility that determines the suitability and quality of wheat flour for different end products.

4 Core factors affecting dough storage protein composition and viscoelastic properties

As mentioned above, gluten functionality is due to the essential role of disulfide bonds, which are formed between sulfhydryl groups of cysteine residue^[79,80]. The cysteine residue are important in the formation of a gluten network and maintaining gluten functionality. Dough elasticity is primarily associated with the polymeric glutenins, which form both intramolecular and intermolecular disulfide bonds, whereas, dough extensibility mainly results from the monomeric gliadins, which form only intramolecular disulfide bonds^[79,81]. It has been reported that intramolecular disulfide bonds form more rapidly than intermolecular disulfide bonds^[12]. According to the distribution of cysteine residues, gluten subunits can be divided into S-poor (ω -gliadin and HMW-GS) and S-rich (α/β -, γ -gliadins and LMW-GS) subunits. The α/β -, γ -gliadins and LMW-GS contain two or four times more cysteine residues than HMW-GS; ω -gliadin contains no cysteine or methionine residues^[79].

Differences in soil sulfur availability may change the proportion of S-poor (ω -gliadin and HMW-GS) and S-rich (α/β -, γ -gliadins and LMW-GS) subunits and thus alter the grain storage protein composition, which may eventually lead to variation in the grain quality^[82–85]. Less sulfur availability in grain leads to an increase of S-poor subunit quantity including ω -gliadin and HMW-GS, and there is a slight reduction in the amount of gliadins and LMW-GS. Therefore, a decreased sulfur content is positively related to an increased ratio of HMW-GS to LMW-GS. These compositional changes could lead to an increased elasticity and a decreased extensibility in gluten functionality^[86,87]. In contrast, high sulfur availability in grain relates to an increased proportion of S-rich subunits, which results in an increased proportion of LMW-GS, $\alpha/\beta/\gamma$ -gliadin and a decreased proportion of HMW-GS and ω -gliadin, and an altered molecular weight distribution^[83,85,87–89]. Ultimately, the effect of high sulfur content will decrease elasticity and simultaneously increase extensibility^[90,91].

Apart from the effects on gluten protein composition, another possible impact of sulfur on dough quality is due to the tripeptide glutathione. Sulfur uptake is generally in the form of sulfate, which is then converted into cysteine that forms disulfide bonds important in maintaining gluten functionality through various steps involving activation and reduction^[92,93]. Furthermore, cysteine is metabolized into glutathione in free reduced and free oxidized (GSSG) forms, as well as in the form of protein-glutathione mixed disulfides^[94]. First, the balance between GSSG and GSH is maintained in favor of oxidants such as ascorbic acid and potassium bromate, acting as electron sources^[95–97]. Free GSH could react with intermolecular disulfide bonds, which results in breakdown of disulfide bonds and damage to the structure of polymeric glutenins that ultimately weakens the elasticity of dough. However, free GSSG can react with SH groups of gluten proteins, which results in release of GSH and damages the linkages of disulfide bonds, ultimately weakening the dough quality^[98,99]. Therefore, an excessively high sulfur supply to wheat may increase the formation of glutathione, which is negatively related to dough quality and tends to decrease wheat processing quality.

Based on the extractability of grain protein fractions in the SDS extraction buffer, grain protein compositions can be divided into SDS-unextractable polymeric protein (UPP; glutenin macropolymer) and SDS-extractable polymeric protein (EPP). The concentration of UPP (~20–40 mg·g⁻¹) in total grain protein is crucial in determining gluten strength and breadmaking quality^[58]. UPP is positively related to the formation of the gluten network which is formed by interlinking disulfide bonds and responsible for dough functionality. The percentage of UPP is a reliable reference for dough quality. The growth environment has less effect on UPP accumulation in the process of grain development. In contrast, EPP has been reported to be influenced by environmental factors^[100,101].

Although the accumulation of polymeric proteins starts as early as 7 d post-anthesis and lasts during the entire period of grain development, the accumulation of UPP fraction only begins at the later stage of grain development (from 30 to 45 d post-anthesis). Yu et al.^[102] found that UPP formation involves peptidyl-prolyl cis-trans isomerase (PPIase) SUMOylation with the assistance of small ubiquitin-related modifier 1 and that high nitrogen availability facilitates this connection. Additionally, luminal binding protein 2 in the endoplasmic reticulum has a similar function to PPIase in the aggregation of protein. The formation of UPP is closely related to the process of moisture loss during grain-filling^[103]. It has also been reported that the significant changes in size distribution of polymers only occurs during the later stage of seed development, which corresponds with the period of a sharp increase in glutenin subunit amount during cell division and enlargement. Glutenin subunits have a large number of free SH groups and are oxidized during water loss, which is also when UPP is formed^[104,105].

5 Conclusions

Extensive studies have been conducted to understand the fundamental mechanisms underlying the effect of gluten on wheat end product quality. The HMW glutenin proportion has the greatest impact on dough quality. The current progress in wheat genome research has made it easy to search for novel gluten genes for wheat quality improvement. Based on the findings of gluten research, in the wheat genomic era, new HMW glutenins identified from wheat relatives and historical landraces with long central repetitive domains that contain high number of consensus hexapeptide and nonapeptide motifs as well as high content of cysteine and glutamine residues should be investigated for their potential to improve wheat end-use quality.

Compliance with ethics guidelines Wujun Ma, Zitong Yu, Maoyun She, Yun Zhao, and Shahidul Islam declare that they have no conflicts of interest or financial conflicts to disclose.

This article is a review and does not contain any studies with human or animal subjects performed by any of the authors.

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